

Lead transfer in the soil-root-plant system in a high contaminated Andean area

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Soil lead is not an essential factor in agricultural production; on the contrary, it is a heavy metal that is highly toxic to the nutritional food system. This study was conducted in 2018 to evaluate the level of lead transfer and bioaccumulation in the soil-root-plant system in high Andean grasslands in a geographic area near La Oroya, in the central Andes of Peru, where large companies have been engaged in mining-metallurgical activities for almost a century, contaminating the air, water, soil and grasslands, reducing the quality of the soil and food and causing damage to the environment and human health. In this context, lead levels were measured in 120 samples of topsoil (0-20 cm), root and shoot of high Andean grasslands in a systematic study, and lead concentrations were determined by the method of flame atomic absorption spectroscopy. No significant differences between soil pH, organic matter content and lead were found among the samples evaluated ($P > 0.05$). Mean Pb concentrations decreased in the order soil $>$ root $>$ shoot ($P < 0.01$). Pb levels in soil, root and shoot of high Andean grasses were 212.36 ± 38.40 , 154.65 ± 52.85 and 19.71 ± 2.81 mg / kg. Pb transfer factors from soil, roots and shoots were 0.74 ± 0.26 and 0.14 ± 0.06 , and Pb bioaccumulation factors from soil, roots and shoots were 0.10 ± 0.03 . Average soil and shoot lead concentrations exceeded the maximum limits of international regulations. Lead in soil was 3.4 times higher than the maximum limit for agricultural soil and in forage was 1.97 times higher than the limit value for this foodstuff. Our findings are important and shocking and show that pastures in the Andean areas of Peru would have high levels of Pb compared to other regions and the world, which could cause socio-economic and health problems in exposed populations.

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Abstract

Soil lead is not an essential factor in agricultural production; on the contrary, it is a heavy metal that is highly toxic to the nutritional food system. This study was conducted in 2018 to evaluate the level of lead transfer and bioaccumulation in the soil-root-plant system in high Andean grasslands in a geographic area near La Oroya, in the central Andes of Peru, where large companies have been engaged in mining-metallurgical activities for almost a century, contaminating the air, water, soil and grasslands, reducing the quality of the soil and food and causing damage to the environment and human health. In this context, lead levels were measured in 120 samples of topsoil (0-20 cm), root and shoot of high Andean grasslands in a systematic study, and lead concentrations were determined by the method of flame atomic absorption spectroscopy. No significant differences between soil pH, organic matter content and lead were found among the samples evaluated ($P > 0.05$). Mean Pb concentrations decreased in the order soil > root > shoot ($P < 0.01$). Pb levels in soil, root and shoot of high Andean grasses were 212.36 ± 38.40 , 154.65 ± 52.85 and 19.71 ± 2.81 mg / kg. Pb transfer factors from soil, roots and shoots were 0.74 ± 0.26 and 0.14 ± 0.06 , and Pb bioaccumulation factors from soil, roots and shoots were 0.10 ± 0.03 . Average soil and shoot lead concentrations exceeded the maximum limits of international regulations. Lead in soil was 3.4 times higher than the maximum limit for agricultural soil and in forage was 1.97 times higher than the limit value for this foodstuff. Our findings are important and shocking and show that pastures in the Andean areas of Peru would have high levels of Pb compared to other regions and the world, which could cause socio-economic and health problems in exposed populations.

Subjects: Environmental Contamination and Soil Science, Ecosystem Science, Environmental impacts

Keywords: Heavy metals, Contaminated soil, Soil pH, Forage quality, Lead bioaccumulation, Smelting, Bioaccumulation factor, Lead in plants, Highland Andes Peru.

Introduction

Lead (Pb) is a toxic metal ($11.4 \text{ g} / \text{cm}^3$) naturally present in the earth's crust in harmless concentrations, usually between 15 and 40 mg / kg (Assi et al., 2016), but is the second most dangerous substance (Fahr et al., 2013). Due to its multiple uses, it is widely used in industry (Wuana & Okieimen, 2011) and in its metallurgical process it produces fine particulate material that travels many kilometers through the air (Suvarapu & Baek, 2017; Martin et al., 2017) and when it exceeds certain limits it geoaccumulates, bioaccumulates and biomagnifies (Lokeshwari & Chandrappa, 2006). It is deposited in water and soil and transferred to plants, animals and humans (Li et al., 2017) reaching toxic levels with serious consequences for ecosystems (Alloway, 2013; Kong, 2014), with adverse effects on plants, animals and humans, especially

40 babies and children (Kryshna & Mohan, 2016; Chirinos-Peinado & Castro-Bedriñana, 2020). It
41 contaminates groundwater and reduces the quality of soil and food (Martin et al., 2017).

42 Anthropogenic Pb released into the atmosphere is deposited and accumulates in the upper soil
43 layer (Bacon, Hewitt & Cooper, 2005), and because of its long biological half-life and high
44 bioaccumulation potential, it is absorbed by grass roots (Nascimento et al., 2014; Hou et al.,
45 2014), generating unsafe Pb-laden foods that harm human health (Li et al., 2005; Castro,
46 Chirinos & Rios, 2016).

47 Soil quality plays an important role in food security by determining the possible composition
48 of forage at the early levels of the food chain (Tóth et al., 2016); therefore, top soil analysis
49 (typically the upper 0-20 cm) is valuable for assessing heavy metal contamination in grasslands
50 (Ekengele, Danala & Zo'o, 2016; Martín et al., 2017).

51 Soil contamination and its potential impact on human health has not been studied extensively
52 in Peru, especially in the high Andean areas, where mining and metallurgical activities are
53 carried out in addition to livestock activities. Elsewhere, such as in Europe, 93.76 per cent of its
54 agricultural land has been found to be safe for food production and only 137,000 km² (6.24 per
55 cent) is estimated to be contaminated by heavy metals and needs to be assessed locally and
56 remedial action taken (Tóth et al., 2016); such information is not available in Peru.

57 Although mining generates the largest percentage of net exports and foreign exchange to
58 Peru, the central highlands may have marked accumulations of Pb in surface soils that are
59 transferred to pastures and other crops. The polymetallic mining-metallurgical center located in
60 the central sierra, which has been in operation since 1992, generated uncontrolled emissions
61 between the 1970s and 1990s, and although some processes were subsequently optimized,
62 reducing Pb emissions to the air, emissions of particulate matter and acid fumes continue to
63 exceed international standards and contaminate the surrounding ecosystems (Álvarez-Berrios et
64 al., 2016). La Oroya is the fifth most contaminated place on the planet (Blacksmith Institute,
65 2007) and in these almost 100 years, the vegetation and soil of the central region of Peru have
66 been contaminated by a series of heavy metals and toxic substances (Alvarez-Berrios et al.,
67 2016; USDA, 2017).

68 A recent study shows that the milk produced in an area close to the metallurgical industry
69 presents quantities of Pb and Cd higher than the maximum permitted levels (Chirinos-Peinado &
70 Castro-Bedriñana, 2020), which makes soil quality an issue of economic and social importance
71 (Kong, 2014) not only for Peru, but for the world.

72 There is great interest in studying the bioaccumulation of heavy metals in forages, since they
73 are the basis of the food chain. From this point of view, our study addresses the bioaccumulation
74 of Pb in high Andean pastures in highly contaminated soils in an area near the city of La Oroya.

75 We evaluated the topsoil, roots and shoots of natural and cultivated pastures in a livestock
76 area between April and September 2018. This study expands our knowledge of the dynamics of
77 Pb transfer in the soil-root-sprout system under highly contaminated conditions.

78 In general, the knowledge of contaminant levels in food is generated in controlled studies,
79 with pre-set amounts of samples in controlled environments during a limited period (Adamse,

80 Van der Fels-Klerx, de Jong, 2017). A strength of our ecological study is that it allows us to
 81 study the problem over time and provides information on the levels of lead in grasslands to
 82 establish monitoring priorities and continue research on the dynamics of lead in the soil.

83

84 **Materials and methods**

85 **Ethical approval**

86 The study protocol was approved by a group of experts appointed by the University General
 87 Research Institute. The institutional authority of the high Andean community where the research
 88 was conducted approved the field study and the soil and pasture sampling.

89

90 **Description of the study site**

91 The research was carried out in the pastures of the Pacha Peasant Community, located in the
 92 central Andes of Peru, department of Junin, province of Yauli (11°32'10" South Latitude and
 93 75°45'20" West Longitude, average altitude 3750 m, minimum and maximum temperature of -
 94 3.1 and 18.2°C), covering 16,564 ha of pastures, of which 13.73 ha are irrigated.

95 The study site is located 20 km from the La Oroya Polymetallic Metallurgical Complex,
 96 which has been in operation since 1922. Figure 1 shows the location map and the distribution of
 97 soil, root and shoot sampling points of cultivated and natural pastures in the two collection
 98 periods.

99 This area was chosen because it is representative of the communities near the fifth most
 100 contaminated city on the planet (Blacksmith Institute, 2007), which due to their agro-ecological
 101 conditions are dedicated to raising cattle, sheep and alpacas.

102 In the area of study, the cattle have approximately 11 ha (hectares) of natural pasture, with the
 103 following species standing out: *Festuca dolichophylla*, *Bromus catharticus*, *Bromus lanatus*,
 104 *Nasella meyeniana*, *Calamagrostis heterophylla*, *Piptochaetium faetertonei* *Nasella publiflora*,
 105 *Asiachnae pulvinata*, *Margaricarpus pinnatus*, *Oenothera multicaulis*, *Trifolium amabile* and
 106 small extensions of associated grasses (6 ha) *Lolium perenne* and *Trifolium repens*, installed 15
 107 years ago and currently have a poor condition.

108 The quantification of Pb in soil, roots and shoots of the high Andean grasses was carried out
 109 in the SAC laboratory of Baltic Control, accredited by the National Institute of Quality of Peru.

110

111 **Figure 1.** Partial map of La Oroya-Peru, research area (3900-4500 m altitude).

112 The image above shows the path from La Oroya Mining-Metallurgical complex.

113 The lower image shows the study site and distribution of sampling points (1 m²/sample).

114 Communal stable: to 20 km from La Oroya City.

115  Sampling area (n = 10) of pastures cultivated - April samples

116  Sampling area (n = 10) of pastures cultivated - September samples

117  Sampling area (n = 10) of natural pastures - April samples

118  Sampling area (n = 10) of natural pastures - September samples

119

120 **Sampling procedures**

121 Considering that most crop roots are present in the upper 20 cm of the soil (Evans, 1978), topsoil
122 sampling (0-20 cm) was carried out at 20 points in the natural grassland area and 20 points in the
123 cultivated grassland area (Figure 1), which were collected in April and September 2018. A total
124 of 120 samples were collected using standardized sampling procedures (Tóth et al., 2016; Li et
125 al., 2016; Martin et al., 2017; Castro-Bedriñana, Chirinos-Peinado, Peñaloza-Fernández, 2020).
126 Soil and grasses of 1 m² were sampled with a stainless-steel shovel, taking approximately 0.5 kg
127 of soil from each and the grasses present (Tóth, Jones, Montanarella, 2013).

128 The soil and grass samples are from the same sampling site (Figure 1). The grasses were
129 divided into root and aerial parts (García-Gallegos et al., 2011; Castro-Bedriñana, Chirinos-
130 Peinado, Peñaloza-Fernández, 2020). All samples were placed in first-use polyethylene bags
131 with zippered closure to be taken to the laboratory.

132

133 **Laboratory analysis**

134 The soil samples were dried at normal temperature, pulverized and sieved on a 100-mesh screen,
135 obtaining a fine and homogeneous powder. The samples were digested using the USEPA 3050B
136 method (SW-846). Roots and shoots were washed with running water and rinsed with deionized
137 water, dried and ground for analysis.

138 Repeated additions of a mixture of concentrated nitric acid (HNO₃) and hydrogen peroxide
139 (H₂O₂) were made to one gram of dry sample. Hydrochloric acid (HCl) was added to the initial
140 digestate and the sample was refluxed. The digestate was diluted to a final volume of 100 ml
141 (USEPA, 1996).

142 To quantify the Pb concentration of the different samples, the standard analysis procedure was
143 followed (Tóth, Jones, Montanarella, 2013), using flame atomic absorption spectroscopy
144 (FLAA), based on the protocol of the AOAC Official Method 975.03 (AOAC, 1990; USEPA,
145 1996). To ensure analytical precision, the blank method, duplicate samples, and the high- and
146 low-range control standard were used for every 15 samples analyzed. For the calibration curve,
147 Sigma-Aldrich Pb standard 986 ± 4 mg / kg was used. The detection limit for Pb was 0.2 mg/kg.
148 The units of concentration of Pb were expressed in mg/kg.

149 To assess the Pb concentration in soil, the international threshold of 60 mg/kg was used
150 (MEF, 2007) and for forage, the maximum Pb limit was 10 mg/kg dry matter, considering that
151 limits in the normal plant range from 0.5 to 10 mg / kg dry matter (Kabata-Pendias & Pendias,
152 2001; Boularbah et al., 2006).

153

154 **Lead transfer factor and bioaccumulation**

155 The transfer factor (TF) represents the potential transmission capacity of heavy metals from the
156 soil to different parts of a plant (Hu et al., 2017). The equations used to estimate the TF of Pb
157 from soil to root (TF_{sr}), from root to shoot (TF_{rs}) and the bioaccumulation from soil to shoot
158 (TB_{ss}) were the following:

159

160

$$TF_{sr} = [Pb\ root]/[Pb\ soil]$$

161

162

$$TFrs = [Pb\ Shoot]/[Pb\ Root]$$

163

164

$$TBss = [Pb\ Shoot]/[Pb\ soil]$$

165

166 **Statistical analysis**

167 To compare the Pb content between soil, root, and shoot samples from natural and cultivated
168 pastures per sampling period, analyses of variance and Tukey tests were performed with a
169 significance level of 5% using SPSS 23. To compare the level of Pb in soil and grass with the
170 maximum permissible limits, "t" tests were performed for a single sample. The values of the
171 maximum Pb limits in soil and grass were 60 and 10 mg/kg, respectively.

172

173 **Results**

174 **Soil properties and Pb content**

175 No significant differences were found between soil pH by sampling period and by type of grass
176 ($P > 0.05$). The pH range was between 5.03 and 7.73, with an average of 6.36, indicating that the
177 soils evaluated are weakly acidic to neutral (Table 1).

178

179 **Table 1.** Average and range of pH, organic matter (OM) and Pb content in the soil in different
180 study periods in an area near the polymetallic smelter in the central Andean region of Peru.

181

182 **Lead concentration in soil, roots and shoots**

183 Table 2 summarizes the results of average Pb concentrations in topsoil (0-20 cm), roots and
184 shoots of high Andean pastures.

185

186 **Table 2.** Lead concentration in soil, roots and shoots of high Andean pastures (mg / kg) in an
187 area near the polymetallic smelter in the central Andean region of Peru.

188

189 **Figure 2.** Average lead concentration in soil, roots and shoots from high Andean pastures in an
190 area near the polymetallic smelter in the central Andean region of Peru. The maximum
191 permissible values of Pb in soil and grass are shown (MEF 2007; Kabata-Pendias & Pendias,
192 2001; Boularbah et al., 2006).

193

194 **Lead transfer and bioaccumulation factors**

195 The TF_{sr} was 0.74 ± 0.26 , with a range between 0.66-0.83 (Table 3, Figure 3); result that differs
196 with the observations of Li et al. (2005) that reports a Pb availability in soils lower than 1%.

197

198 **Table 3.** Lead transfer factors in the soil-root-sprout system in high Andean pastures in an area
199 near the polymetallic smelter in the central Andean region of Peru.

200

201 **Figure 3.** Lead transfer factors in soil-root-shoots system from natural and cultivated pastures in
202 an area near the polymetallic smelter in the central Andean region of Peru.

203

204 The TBss was 0.10 ± 0.03 , with a range between 0.09-0.10, a result that shows that the root
205 bioaccumulates the most Pb from the soil. The TFRs was 0.14 ± 0.06 , with a range between 0.12-
206 0.16; in other words, the maximum transference percentage was 16%, leaving 84% of Pb
207 concentrated in the root.

208

209 **Discussion**

210 **Soil properties**

211 The soils were medium to thin, loam to sandy loam (USDA, 2017), neutral to slightly acidic in
212 pH. These edaphic characteristics are generalized in the soils of the central highlands of Peru.
213 Unmodified or weakly modified soils are poor in nutrients and maintain their nutritional status
214 by relying on natural biogeochemical processes (McLaughlin et al., 2000); these soils are
215 especially sensitive to the effects of heavy metal contamination.

216 The OM and Pb contents in the soil were similar by type of grass and sampling period ($P >$
217 0.05), so the degree of solubility and bioavailability of Pb for plants would also be similar
218 throughout the year (Lokeshwari & Chandrappa, 2006; Nas & Ali 2018), maintaining chronic
219 contamination.

220 The results are indicative of the poor OM of the high Andean soils of central Peru; and,
221 although Pb has a weak capacity to inhibit the decomposition of matter, due to its low solubility
222 and persistent toxicity (Enya et al., 2020), improvements should be implemented so that the soil
223 has at least 5% of OM, recommending fertilization with the manure of high Andean cattle.

224

225 **Lead concentration in soil, roots and shoots**

226 The soils of La Oroya have received contamination since the smelter industry began operations
227 in 1922 (Chirinos-Peinado and Castro-Bedriñana, 2020). For 2007 and 2008, it reported an
228 average concentration of $PM_{2.5}$ particles in La Oroya and a study area of 32.4 and 20.3 $\mu\text{g} / \text{m}^3$,
229 which exceed the ECA $PM_{2.5} = 15 \mu\text{g}/\text{m}^3$ (MINAM, 2008) and 52.3 and 42.4 $\mu\text{g} / \text{m}^3$ of PM_{10}
230 ($FSS PM_{10} = 50 \mu\text{g} / \text{m}^3$), which is deposited in the soil, then assimilated by plants and animals,
231 affecting human health at the end of the food chain (Kryshna and Mohan, 2016; Li et al, 2017),
232 since under similar conditions a 5% increase in mortality for every 50 $\mu\text{g} / \text{m}^3$ of Pb in the air is
233 reported (WHO, 2006).

234 Pb content decreased in the order of soil > root > shoot ($P < 0.01$), which is consistent with other
235 findings (Peláez-Peláez, Bustamante & Gómez, 2016). Lead concentrations in the soil root shoot
236 (Figure 3) are above the maximum allowable levels, 60 mg/kg in the case of soil and 10 mg/kg in
237 the case of shoots. The enrichment of Pb in the grass roots would act as a barrier to the
238 translocation of heavy metals to the aerial part of the grasses.

239

240 **Lead concentration in the soil**

241 The current result is much higher than the world average (25 mg / kg) and it seems likely that Pb
242 in the soils of the central Peruvian highlands are at the upper end on a global scale, as identified
243 above (Castro-Bedriñana, Chirinos-Peinado, Peñaloza-Fernández, 2020).

244 The reference value for mean soil Pb concentrations is 60 mg / kg (MEF, 2007), and in the
245 present study, concentrations for natural and cultivated pasture soils were 219.12 and 205.6
246 0.016 mg / kg. Comparing the mean Pb concentration in the soil of the study area (212.36 mg /
247 kg) with the maximum limit for agricultural soils (MEF, 2007), it was observed that the mean Pb
248 concentration was 3.54 times higher than the maximum limit for agricultural use and pasture
249 production ($P < 0.01$), with implications for South American cattle, sheep and camelids and
250 consequently for public and environmental health (Chirinos-Peinado and Castro-Bedriñana,
251 2020). Similarly, it was 4.35 times higher than the limit for forage cultivation recommended by
252 Kabata-Pendias and Pendias (2001) of 50 mg Pb / kg of soil; it was also higher than other
253 international references (Peláez-Peláez, Bustamante & Gómez, 2016) that indicate a range of 7.5
254 to 135 mg / kg for agricultural soils in the United States of America, and those indicated by the
255 Peruvian Ministry of Agriculture, of 70 mg / kg (MINAM, 2017). These findings are important
256 and shocking, and could even have legal significance, since mining-metallurgical companies
257 would be altering environmental, animal and human health, and would also be causing damage
258 to the economic systems that affect people exposed to these conditions.

259 The results of this study are also well above what was determined in the Auckland region of New
260 Zealand, with a Pb content between 1.5 and 65 mg / kg (ARC, 2007) indicating that their soils
261 are moderately contaminated reporting a Pb content of 11.4 mg / kg in the last 15 years
262 (Auckland Council, 2015).

263 Scientific information indicates that Pb concentrations in the soil between 10 and 30 mg / kg do
264 not affect plant growth (Mlay and Mgumia, 2010); currently, the concentrations are of high risk
265 to human health because they are incorporated into crops grown in this lead-laden soil.

266 Pb concentrations in soil of 100-400 mg / kg are considered toxic, and in this study the values
267 were within this range, being toxic to plants, animals and their products, so it is recommended to
268 monitor the impact of soil contamination on their quality and crops (Morera et al., 2013).

269 These results serve as information to evaluate the load of Pb in the environment and its potential
270 to enter the food chain (Hou et al., 2014), and the mining-metallurgical industry should control
271 and reduce its emissions, which can remain in the environment for 150 to 5000 years (Saxena et
272 al., 1999).

273 In Peru, the problem of contaminated soils and the products obtained from them have been
274 neglected, which forces us to think about an integrated management of soils, which includes their
275 evaluation, use planning, territorial ordering and decontamination strategies to apply
276 environmental quality safety.

277

278 **Lead concentration in the root**

279 The average Pb content in the roots of natural and cultivated grasses was between 125.35
280 and 183.96 mg / kg. This high Pb content would force the plants to develop tolerance
281 mechanisms in their root system, avoiding the higher Pb absorption and its retention in the
282 vacuole, affecting the growth and branching pattern of the roots and the rate of Pb translocation
283 to the aerial parts of the plant (Fahr et al., 2013). It was observed that the Pb transfer factor from

284 the root to the shoot is relatively low, a result that suggests that the root would act as a Pb
285 accumulating organ protecting the edible part of the plant (table 2).

286 In this highly contaminated environment, the roots develop various mechanisms to attenuate
287 the absorption and transfer of Pb to the aerial parts of the plant and reduce its harmful effects
288 (Fahr et al., 2013). In some plants, there is callus formation between the plasma membrane and
289 the cell wall that acts as a barrier against metals (Samardakiewicz et al., 2012; Pirselova et al.,
290 2012). In a study in tomato, higher concentrations of Pb in the roots are also reported compared
291 to the other products (Akinci, Akinci & Yilmaz, 2010).

292 In most plants, about 90% of total Pb accumulates in the root (Kumar et al. 1995) and the
293 cell walls can immobilize Pb ions (Inoue et al. 2013). In the present study, roots accumulated
294 87.6% of Pb from the plant and 12.4% of Pb was translocated to the aerial part of the grasses.

295 The roots of high Andean grasslands tolerate and accumulate high levels of Pb and the
296 highest overall rate of Pb accumulation occurred in the roots rather than in the shoots
297 (Nascimento et al. 2014), suggesting that, under conditions of chronic contamination, it is a
298 survival mechanism of the Andean pastures studied.

299

300

301 **Lead concentration in shoots**

302 In the study area, the pastures consumed by cattle showed a bioaccumulation of Pb above the
303 permissible limits, which could generate problems of biomagnification in the food chains,
304 damaging animal and human health and productivity (Chirinos-Peinado and Castro-Bedriñana,
305 2020). The average Pb content in shoots was 1.97 times higher than the limit value of 10 mg / kg
306 for forages ($P < 0.01$) (Boularbah et al., 2006; Kabata-Pendias & Mukherjee, 2007) and 6.57
307 times higher than the critical value proposed for vegetables 0.05-3.0 mg / kg (Tokalioglu, Kartal
308 & Gunis, 2000).

309 Normal Pb concentrations in mature leaf tissues of various plant species are between 5 and 10
310 mg / kg, considering a toxic range of 30 to 300 mg / kg (Kabata-Pendias & Mukherjee, 2007).
311 Other proposals for an upper limit of Pb in pastures suggest 30 mg / kg of dry matter (Mlay and
312 Mgumia, 2010; Adamse et al., 2017); however, this concentration in the long term may slow
313 down plant growth and affect their biochemical functions (Sharma and Dubey, 2005); other
314 authors propose as "normal level" values between 2 and 5 mg / kg (Robinson et al, 2008); even
315 the Codex Alimentarius (ONU/FAO, 2018) indicates a maximum permissible limit of Pb in
316 cattle feed of 0.3 mg / kg. The rules and regulations are increasingly strict, so recently the CDC,
317 in only 8 years, has reduced the critical level of Pb in infants, from 10 to 2 ug/dL of blood
318 (Rădulescu & Lundgren, 2019).

319 In the current study, no differences were found between Pb concentrations in natural and
320 cultivated pastures, but they are higher than those reported in other regions of the country and the
321 world (Longhurst, Roberts and Waller, 2004; Johnsen and Aaneby, 2019); they are higher than

322 the recommended guidelines for human health due to the risk of bioaccumulation in livestock
323 products (Chirinos-Peinado and Castro-Bedriñana 2020).

324 The current results are slightly higher than those found in 31 species of *Brachiaria* in places
325 around the refineries, with values between 9.8 and 16.0 mg / kg; in places around the exploration
326 wells the values were between 9.7 and 13.2 mg / kg, with the highest concentrations recorded
327 near the source of emission (Pelaez-Pelaez, Bustamante & Gomez, 2016).

328 In soils contaminated with Pb slag, levels of 209-899 (425 ± 79.0) mg / kg are reported, while
329 in the control areas the average was 1.24 mg / kg (Ogundiran et al., 2012). In sites close to
330 metallurgical activities, the Pb in forage was 29.06 ± 11.32 mg / kg (Swarup et al., 2005) while,
331 in non-industrialized sites, the average was 2.08 ± 0.22 mg / kg. In this study, the average (19.71
332 ± 2.81 mg / kg) is attributed to sustained contamination from mining-metallurgical activity in the
333 central sierra of Peru, constituting a permanent threat to Andean livestock (Iqbal et al., 2015).

334 In contaminated soils where there is cultivated pasture composed of *Trifolium alexandrinum*,
335 *Brassica campestris* and *Avena sativa*, average Pb concentrations between 36.85 and 60.21 mg /
336 kg are reported (Iqbal et al., 2015), higher values than those found in this study. In New
337 Zealand's pastures, values between 4.4 and 26.8 mg / g (average 10.6 mg / kg) are reported
338 (Martin et al., 2017) and between 6 and 16 mg / kg, depending on soil type (Longhurst, Roberts
339 and Waller et al., 2004).

340 In practice, higher concentrations of Pb in forage roots are desirable so that grass shoots can
341 be used as animal feed; however, the Pb levels determined in this study indicate that caution
342 should be exercised when feeding animals with these grasses because they exceeded the 10
343 mg/kg forage threshold (Boularbah et al., 2006; Kabata-Pendias and Pendias, 2001).

344

345 **Lead transfer in the soil root and bioaccumulation in the shoots**

346 The Pb content of the soil is transferred and bioaccumulates in the edible parts of the forage,
347 being a direct route for its incorporation into the food chain, causing damage to soil
348 microorganisms, plants, animals and humans (Harmanescu et al., 2011; Alloway, 2013;
349 Chirinos-Peinado and Castro-Bedriñana, 2020).

350 Under high contamination conditions, the transfer factor (TF) from soil to plant roots
351 averaged 0.74 ± 0.26 (range: 0.54-0.94), a result that would show that the root absorbs and
352 bioaccumulates a large part of the Pb present in the soil and retains it, transferring it to a lesser
353 extent to the aerial part of the plant; however, the shoots had a high Pb content.

354 The determination of the BF of the heavy metals in the shoots in relation to the total soil
355 content is a suitable method to quantify the bioavailability of the metals. Pb has a lower BF than
356 other metals, as it is bound to the soil colloids, being less bioavailable (Violante et al., 2010).
357 The BF of Pb from the soil colloids was slightly above the range of values of 0.01 to 0.10 for
358 soils with 45 to 90 mg/kg Pb (Kloke, Sauerbeck and Vetter, 1984). In our case, the mean soil Pb
359 content was 212.36 mg/kg and the BF range was 0.08 to 0.12; a result that indicates that the

360 highest proportion of Pb in natural and cultivated grasses remains concentrated in the root
361 (Zhang et al., 2013; Song et al., 2019).

362 The BF in our study was below the average recorded for spinach, lettuce, carrots and radishes
363 grown on soils containing 100 mg/kg Pb (Intawongse & Dean, 2008).

364 Our study shows that the mining and metallurgical activities carried out in the central
365 highlands of Peru for almost a century have led to serious problems of environmental
366 contamination in the root and pasture system of the soil used for cattle ranching in the central
367 Andes of Peru; therefore, this is a critical problem that must be resolved (Akhtar et al., 2015; Nas
368 and Ali, 2018).

369

370 **Implications for central Peru**

371 Our findings are important and impressive and demonstrate that the grasslands in the central
372 highlands of Peru would have high concentrations of heavy metals compared to other parts of the
373 country and the world, and could lead to undesirable economic and social outcomes, and
374 regulations should be put in place to mitigate and correct this problem.

375 The data of this study evidence the agro-food production in substrates contaminated by Pb in the
376 areas near the mining-metallurgical industry and if these activities do not improve their
377 technological processes and implement strict environmental adaptation programs, the chronic
378 bioaccumulation rate of Pb could continue with serious effects on environmental and human
379 health, causing damage to the economic systems that affect the exposed populations. A new
380 Peruvian guideline for Pb and other heavy metals levels in soil, forage and food could help to
381 minimize the adverse effects of contamination.

382

383

384 **Conclusions**

385 The mining-metallurgical activity developed in central Peru during almost a century has had an
386 impact on the high concentration of Pb in the upper soil layer and in natural and cultivated
387 pastures of the Peruvian Andes. The average concentrations of lead in the soil, roots and shoots
388 exceeded the maximum limits of international regulations. Lead in soil was 3.4 times higher than
389 the maximum limit for agricultural soil and in forage was 1.97 times higher than the limit value
390 for forage.

391 The study provides comparable information on the Pb content in the soil-root-plant system of
392 pastures used to feed livestock raised in areas near polymetallic mining-metallurgical activity in
393 the high central Andes of Peru.

394 This study is one of the first to evaluate the content and transfer of Pb in the soil-root-plant
395 system in highly contaminated pastures, and the findings are shocking and could lead to socio-
396 economic problems in exposed populations.

397

398 **Acknowledgements**

399 The authors are grateful to the president of the Paccha-La Oroya Community, who authorized
400 and signed the consent form to carry out the field phase of the study. Special thanks to Yauli-La
401 Oroya Agricultural Agency, who facilitated contact with the farming community.

402

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Table 1 (on next page)

Average and range of pH, organic matter (OM) and Pb content in the soil in different study periods in an area near the polymetallic smelter in the central Andean region of Peru.

1

2 **Table 1.** Average and range of pH, organic matter (OM) and Pb content in the soil in different
3 study periods in an area near the polymetallic smelter in the central Andean region of Peru.

Period	pH	OM (%)	Pb (mg/kg)
Dry	6.34 [5.03-7.47]	3.89 [3.15-4.57]	206.91 [125.18-269.93]
Rain	6.38 [5.08-7.73]	3.93 [3.11-4.53]	217.81 [131.76-284.13]
Average	6.36 [5.03-7.73]	3.91 [3.11-4.57]	212.36 [125.18-284.13]

4

5 There are no statistical differences between average values per period ($P > 0.05$)

6

Table 2 (on next page)

Lead concentration in soil, roots and shoots of high Andean pastures (mg / kg) in an area near the polymetallic smelter in the central Andean region of Peru.

1
 2 **Table 2.** Lead concentration in soil, roots and shoots of high Andean pastures (mg / kg) in an area
 3 near the polymetallic smelter in the central Andean region of Peru.

	Natural pasture ¹		Cultivated pasture ²		All pastures	
	Average	95% CI	Average	95% CI	Average	95% CI
Soil	219.12	201.41-236.77	205.60	187.95-223.24	212.36a	200.08-224.64
Root	147.71	125.35-170.06	161.60	139.25-183.96	154.65b	137.75-171.56
Shoot	20.21	18.92-21.49	19.21	17.92-20.49	19.71c	18.81-20.60

4
 5 a, b, c Average Pb contents: soil > root > shoot, vary statistically ($P < 0.01$)

6 ¹ Natural grass composed of *Festuca dolichophylla*, *Piptochaetium faetertonei*, *Bromus*
 7 *catharticus*, *Bromus lanatus*, *Calamagrostis heterophylla*, *Nasella meyeniana*, *Nasella*
 8 *publiflora*, *Asiachnae pulvinata*, *Margaricarpus pinnatus*, *Oenothera multicaulis* y *Trifolium*
 9 *amabile*.

10 ² Associated pasture composed of *Lolium perenne* and *Trifolium repens*.

Table 3 (on next page)

Lead transfer factors in the soil-root-sprout system in high Andean pastures in an area near the polymetallic smelter in the central Andean region of Peru.

1 **Table 3.** Lead transfer factors in the soil-root-sprout system in high Andean pastures in an area
 2 near the polymetallic smelter in the central Andean region of Peru.

	Natural pasture ¹		Cultivated pasture ²		All pastures	
	Average	95% CI	Average	95% CI	Average	95% CI
Soil-root	0.68	0.57-0.79	0.81	0.69-0.92	0.74a	0.66-0.83
Root-shoot	0.15	0.13-0.18	0.14	0.11-0.16	0.14b	0.12-0.16
Soil-shoot	0.10	0.08-0.11	0.10	0.09-0.11	0.10c	0.09-0.11

3
 4 a, b, c, Average Pb Transfer Factor: soil-root > root-shoot > soil-shoot ($P < 0.01$).

5 ¹ Natural grass composed of *Festuca dolichophylla*, *Piptochaetium faetertonei*, *Bromus*
 6 *catharticus*, *Bromus lanatus*, *Calamagrostis heterophylla*, *Nasella meyeniana*, *Nasella*
 7 *publiflora*, *Asiachnae pulvinata*, *Margaricarpus pinnatus*, *Oenothera multicaulis* y *Trifolium*
 8 *amabile*.

9 ²Associated pasture composed of *Lolium perenne* and *Trifolium repens*.

10

11

Figure 1

Partial map of La Oroya-Peru, research area (3900-4500 m altitude). The image above shows the path from La Oroya Mining-Metallurgical complex.

The lower image shows the study site and distribution of sampling points (1 m²/sample).
 Communal stable: to 20 km from La Oroya City. Sampling area (n = 10) of pastures cultivated - April samples
 Sampling area (n = 10) of pastures cultivated - September samples
 Sampling area (n = 10) of natural pastures - April samples
 Sampling area (n = 10) of natural pastures - September samples



Figure 2

Average lead concentration in soil, roots and shoots from high Andean pastures in an area near the polymetallic smelter in the central Andean region of Peru.

The maximum permissible values of Pb in soil and grass are shown (MEF 2007; Kabata-Pendias & Pendias, 2001; Boularbah et al., 2006).

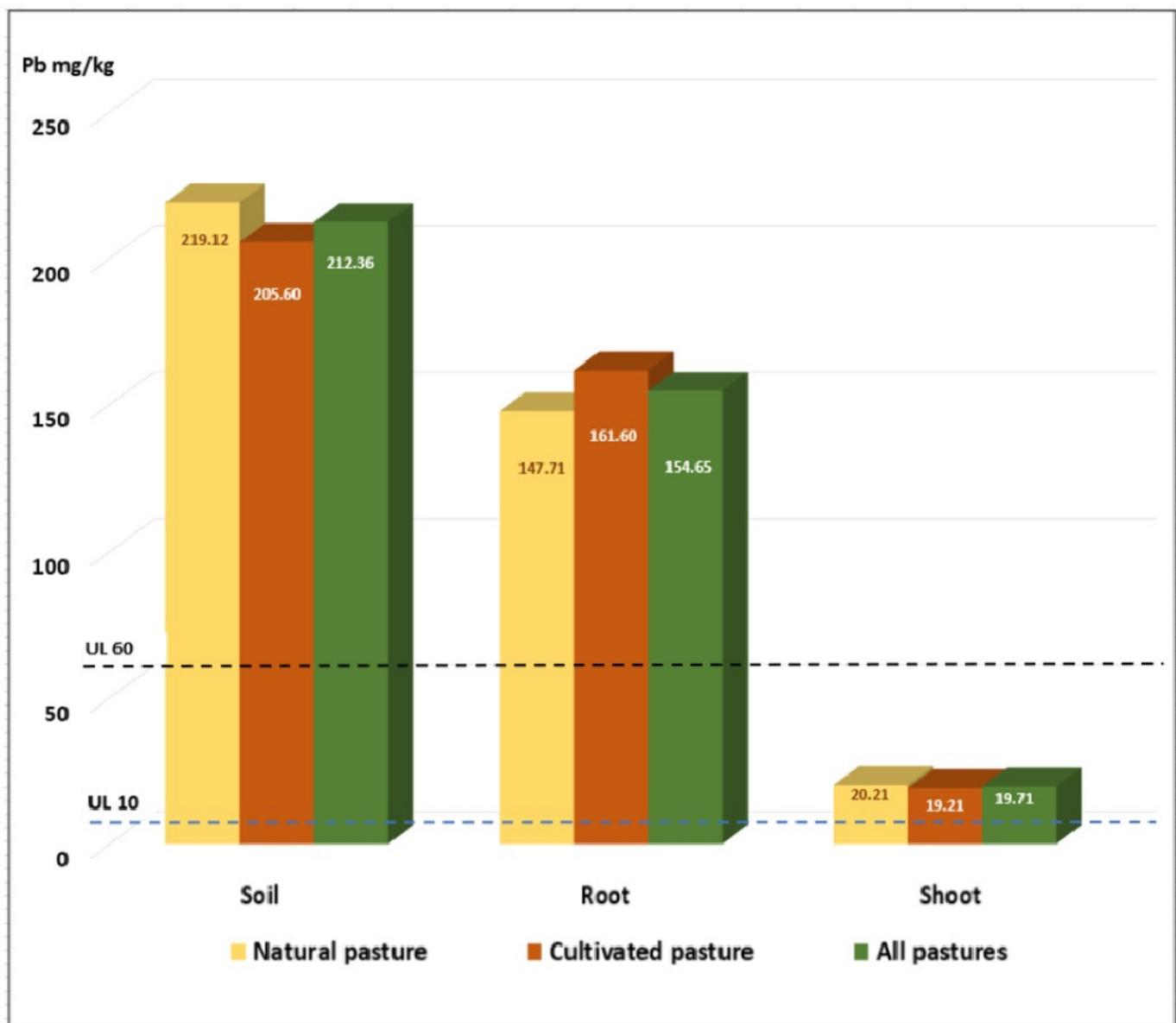


Figure 3

Lead transfer factors in soil-root-shoots system from natural and cultivated pastures in an area near the polymetallic smelter in the central Andean region of Peru.

